



Appl. Serial No. 10/090,798  
Attorney Docket No. NC83202

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re the patent application of: Amanda S. Schilling et. al.

Serial Number: 10/090,798 Art Unit: 1651  
Filed: 03/06/2002 Examiner: Srivastava, Kailash C.

Title: **APPLICATION OF GERMINATION SOLUTION IMPROVED EFFICACY OF BIOLOGICAL DECONTAMINATION**

**DECLARATION OF AMANDA S. SCHILLING, M.S. UNDER RULE 132**

Assistant Commissioner for Patents  
Washington, D.C. 20231

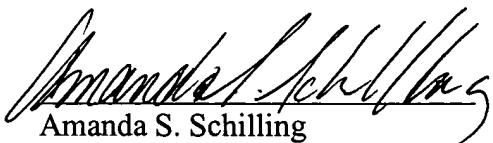
Sir:

1. I, AMANDA S. SCHILLING, of 70 King Henry Court, Fredericksburg, Virginia 22406, am an original and joint inventor of the subject matter which is claimed in U.S. Patent Application No. 10/090,798 which was filed on March 6, 2002, and is entitled APPLICATION OF GERMINATION SOLUTION IMPROVED EFFICACY OF BIOLOGICAL DECONTAMINATION. I am presently employed by the United States Navy, the Assignee of the present invention, as Scientist. I have been an employee of the United States Navy for four (4) years. My educational background includes both B.S. and M.S. degrees in Crop & Soil Environmental Sciences from Virginia Tech of Blacksburg, VA.
2. I have spent four (4) years researching and investigating biological decontamination solutions.
3. The purpose of the instant Declaration is to set forth comparative data which shows the novel

and unobvious use of the combination of dipicolinic acid and calcium ions for rapid decontamination of biological spores.

4. Various prospective germination initiators were tested. Test procedures included treating the spores with one milliliter of a liquid germination initiator for different periods of time. At the end of the set period of time, samples were heated in a water bath at 60°C for 15 minutes. Samples were then plated according to standard microbiological practices. As one of the earliest indicators of germination is loss of heat resistance, the difference between the number of spores before and after heating provided an indication of the percent germination.
5. The attached table, Table 1, shows the percent germination induced as determined by increased heat sensitivity after *Bacillus globigii* spores were treated with various germination initiators for varying periods of time.
6. The designated Germination Initiators listed in Table 1 are: DPA = dipicolinic acid; Ca = calcium; PBS = Phosphate Buffered Saline; 2X YT = 1.6 g tryptone, 1.0g yeast extract and 1.0g sodium chloride in 100 ml sterile water; Germination Buffer = 10mM L-alanine, 200 mM potassium chloride, and 20 mM Tris[hydroxymethyl]-aminomethane hydrochloride); Activated Broken Spores = spores that were treated with 2X Luria-Bertani (LB) Broth for 120 min, then pelleted and washed, and finally, broken apart by a beadbeater.
7. As seen in Table 1, the combined use of dipicolinic acid and calcium ions provides increased spore sensitivity to heat suggesting the induction of more rapid spore germination than other germinants.

8. As seen in Table 1, only combinations of dipicolinic acid and calcium ion provided superior rapid germination. It was found that not all concentrations of dipicolinic acid and calcium ions were superior to other prospective germinants, as seen for DPA-Ca (0.6 mM) after 15 minutes having a germination (%) of 67. Additionally, the use of other germinants with the dipicolinic acid/calcium ions appeared to be detrimental to the efficient germination resulting from the dipicolinic acid/calcium ions, e.g., 2X LB + Ca-DPA 60 mM having a 98% germination compared to DPA-Ca (60 mM) having a 100% germination (both at 15 minutes).
9. The data in Table 1 demonstrates that, for rapid decontamination, the application of a given strength of dipicolinic acid and calcium ions is needed, as specified by the present patent application.
10. I further declare that all statements made herein of my knowledge are true, and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful and false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United states Code and that such willful false statements may jeopardize the validity of the present patent application or any patent issued thereon.



Amanda S. Schilling

2 June 2004  
Date



TABLE 1

Time	Germination Initiator	Germination (%)
15 min	Na <sub>2</sub> CO <sub>3</sub>	0.00
15 min	50x PBS	0.00
15 min	1x PBS	0.00
15 min	10x PBS	0.00
15 min	0.5mL Na <sub>2</sub> CO <sub>3</sub> + LB 0.5mL	2.67
15 min	0.1mg/mL alanine	8.84
15 min	10mM glucose	21.26
15 min	0.1x PBS	23.90
15 min	10mM alanine	26.25
15 min	2XYT - no NaCl	26.60
15 min	4XYT - no NaCl	35.15
15 min	2XYT	37.00
15 min	4XYT	40.86
15 min	2XLB	53.95
15 min	Germination Buffer	55.18
15 min	20mM alanine + 2XLB	56.51
15 min	2XYT + 40mM alanine	58.46
15 min	2XYT + 4mM alanine	61.49
15 min	2XYT + 20mM alanine	62.45
15 min	2XYT + 10mM alanine	65.34
15 min	DPA-Ca (0.6 mM)	67.17
15 min	LB	67.19
15 min	10mM alanine + 2XLB	73.95
15 min	Heat Shocked 2XLB + 20mM ala	77.94
15 min	2X LB +Ca-DPA 60 mM	98.44
15 min	DPA-Ca (30 mM)	98.79
15 min	DPA-Ca (15 mM)	98.90
15 min	DPA-Ca (6 mM)	98.95
15 min	Ca-DPA 30 mM	99.97
15 min	DPA-Ca (60 mM)	100.00
30 min	10mM alanine + 2XLB	76.20
30 min	20mM alanine + 2XLB	80.33
60 min	10mM alanine	34.16
60 min	Germination Buffer	74.18
60 min	LB	77.48
60 min	20mM alanine + 2XLB	96.35
120 min	2XLB + 20mM ala + 5% Na <sub>2</sub> CO <sub>3</sub>	12.82
120 min	20 mM alanine	15.47
120 min	Activated Broken Spores	38.48
120 min	10mM alanine	64.41
120 min	LB	92.69
120 min	Germination Buffer	93.08
120 min	2XLB + 20mM ala + 0.2M KCl + 0.02M Tris HCl	94.88
120 min	4XYT - no NaCl	95.21
120 min	2XLB + 20mM alanine	95.27
120 min	Heat Shocked 2XLB + 20mM alanine	95.37
120 min	4XYT	95.50
120 min	2XLB	95.72
120 min	2XYT - no NaCl	96.00
120 min	10mM alanine + 2XLB	96.37
120 min	2XYT + 4mM alanine	96.42
120 min	2XYT + 40mM alanine	96.44
120 min	10mM alanine + 2XYT	96.76
120 min	Supernatant 2XLB + 20mM alanine	96.77
120 min	2XYT + 20mM alanine	97.20
120 min	20mM alanine + 2XLB	98.79
120 min	Ca-DPA 60 mM	99.83
120 min	60 mM alanine-Ca-DPA	99.96
120 min	60 mM alanine-Ca-DPA	99.99
120 min	60 mM Ca-DPA	99.99